OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Viral RNA Kit

R6874-00	5 preps
R6874-01	50 preps
R6874-02	200 preps

September 2012

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Viral RNA Kit

Table of Contents

Manual Revision: September 2012

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Introduction and Overview

E.Z.N.A.® Viral RNA Kit is designed for isolation of viral RNA from cell-free fluids such as plasma, serum, urine, and cell culture supernatant. The procedure completely removes contaminants and enzyme inhibitors, making viral RNA isolation fast, convenient, and reliable. This kit has been tested for isolating viral nucleic acids from hepatitis A, C, and HIV. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and gram negative bacteria. RNA purified using the E.Z.N.A.® Viral RNA method is ready for applications such as RT-PCR*.

The E.Z.N.A.® Viral RNA Kits use the reversible binding properties of HiBind® matrix, a silica-based, time-saving material. Combined with the speed of mini column spin technology or vacuum manifold, multiple samples can be processed at the same time. The sample is lysed under highly denaturing buffer conditions so that RNases are inactivated, and the intact viral RNA is protected from degradation. After adjusting the buffer conditions, the samples are transferred to the HiBind® RNA Mini Column. With brief centrifugation or vacuum, the samples pass through the column and the viral RNA binds to the HiBind® matrix. After two wash steps, purified viral RNA is eluted with RNase-free water.

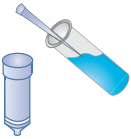
E.Z.N.A.® Viral RNA Kits are not designed to separate viral RNA from cellular RNA and DNA. It will purify both in parallel if they present in the sample. Acellular body fluids are recommended.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

Centrifugation Protocol

Add QVL Lysis Buffer and Lyse

Add Ethanol



Apply Sample to Column

Wash 3X

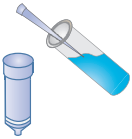
Dry

Elute

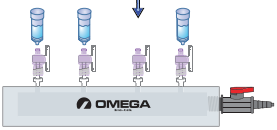
Vacuum Protocol

Add QVL Lysis Buffer and Lyse

Add Ethanol



Apply Sample to Column



Wash 3X

Dry

Elute

Kit Contents

Product	R6874-00	R6874-01	R6874-02
Purifications	5	50	200
HiBind® RNA Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
QVL Lysis Buffer	5 mL	30 mL	120 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
VHB Buffer	2.2 mL	15 mL	66 mL
Carrier RNA (Poly A)	50 µg	320 µg	1600 µg
DEPC Water	1.5 mL	10 mL	30 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Viral RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Carrier RNA must be stored at -20°C. All other components can be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in QVL Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

1. Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R6874-00	20 mL
R6874-01	48 mL
R6874-02	200 mL

2. Add DEPC Water to the tube of lyophilized Carrier RNA as follows. Dissolve the Carrier RNA completely, aliquot, and store at –20°C. Do not freeze–thaw the aliquots more than three times.

Kit	DEPC Water to be Added
R6874-00	50 µL
R6874-01	320 µL
R6874-02	1.6 mL

3. Dilute VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R6874-00	2.6 mL
R6874-01	19.1 mL
R6874-02	84 mL

Recommended Settings

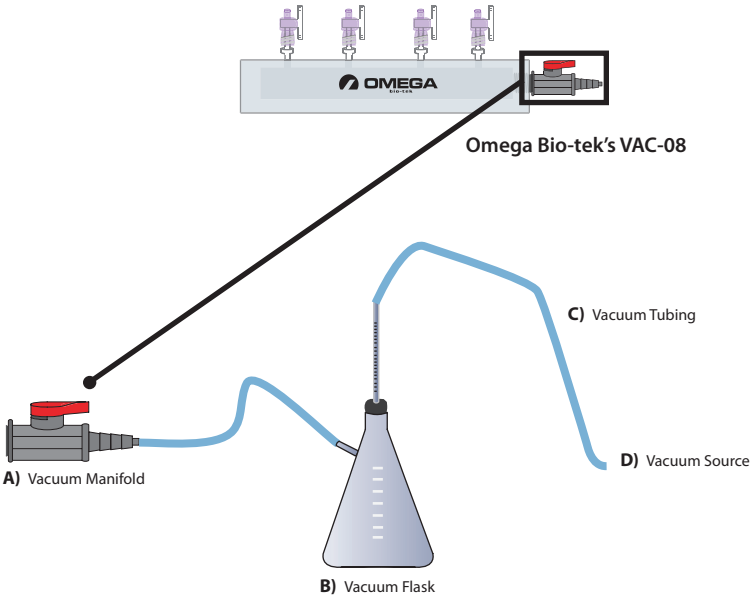
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup:



E.Z.N.A.® Viral RNA Kit Protocol - Centrifugation Protocol

All steps should be performed at room temperature.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- Sterile nuclease-free 1.5 mL microcentrifuge tubes
- Sterile nuclease-free pipette tips

Before Starting:

- Equilibrate samples and QVL Lysis Buffer to room temperature
- Prepare RNA Wash Buffer II, VHB Buffer, and Carrier RNA according to the Preparing Reagents section on Page 5

1. Prepare a master mix of QVL Lysis Buffer and Carrier RNA according to the table below.

   Note: QVL Lysis Buffer and Carrier RNA are stable at 2-8°C for 48 hours. When stored at 2–8°C, this mixture forms a precipitate that must be redissolved before use. Warm the mixture to 80°C. Do not warm for more than 5 minutes.

Number of Preps	Amount of QVL Lysis Buffer (mL)	Amount of Carrier RNA (µL)
1	0.56	5.6
2	1.12	11.2
3	1.68	16.8
4	2.24	22.4
5	2.80	28.0
6	3.36	33.6
7	3.92	39.2
8	4.48	44.8
9	5.04	50.4
10	5.60	56.0

2. Add 500 µL QVL Lysis Buffer into a 1.5 mL microcentrifuge tube (not provided).

3. Add 150 µL plasma, acellular body fluid, cell culture supernatant, or urine to the QVL Lysis Buffer. Vortex for 30 seconds to mix thoroughly.

4. Let sit at room temperature for 5-10 minutes.

5. Centrifuge briefly to collect any liquid droplets from the lid.

6. Add 350 µL 100% ethanol. Vortex for 30 seconds to mix thoroughly.

7. Centrifuge briefly to collect any liquid droplets from the lid.

8. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube (provided).

9. Transfer 750 µL sample (including any precipitate) to the HiBind® RNA Mini Column.

10. Centrifuge at maximum speed (≥13,000 x *g*) for 15 seconds.

11. Discard filtrate and reuse the collection tube.

12. Repeat Steps 9-11 until all the sample has been transferred to the HiBind® RNA Mini Column.

13. Transfer the HiBind® RNA Mini Column to a new 2 mL Collection Tube.

14. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

15. Centrifuge at maximum speed for 15 seconds.

16. Discard the filtrate and the collection tube.

17. Transfer the HiBind® RNA Mini Column to a new 2 mL Collection Tube.

18. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

19. Centrifuge at maximum speed for 15 seconds.

20. Discard filtrate and reuse the collection tube.

21. Repeat Steps 18-20 for a second RNA Wash Buffer II wash step.

22. Centrifuge the empty HiBind® RNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

23. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

24. Add 20-50 µL DEPC Water directly to the center of column matrix.

25. Centrifuge at maximum speed for 1 minute.

26. Store RNA at -70°C.

E.Z.N.A.® Viral RNA Kit Protocol - Vacuum Protocol

All steps should be performed at room temperature.

Materials and Equipment to be Supplied by User:

- Vacuum manifold with standard luer adaptor
- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- Sterile nuclease-free 1.5 mL microcentrifuge tubes
- Sterile nuclease-free pipette tips

Before Starting:

- Equilibrate samples and QVL Lysis Buffer to room temperature
- Prepare RNA Wash Buffer II, VHB Buffer, and Carrier RNA according to the Preparing Reagents section on Page 5

1. Prepare a master mix of QVL Lysis Buffer and Carrier RNA according to the table below.

   Note: QVL Lysis Buffer and Carrier RNA are stable at 2-8°C for 48 hours. When stored at 2–8°C, this mixture forms a precipitate that must be redissolved before use. Warm the mixture to 80°C. Do not warm for more than 5 minutes.

Number of Preps	Amount of QVL Lysis Buffer (mL)	Amount of Carrier RNA (µL)
1	0.56	5.6
2	1.12	11.2
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6	3.36	33.6
7	3.92	39.2
8	4.48	44.8
9	5.04	50.4
10	5.60	56.0

2. Add 500 µL QVL Lysis Buffer into a 1.5 mL microcentrifuge tube (not provided).

3. Add 150 µL plasma, acellular body fluid, cell culture supernatant, or urine to the QVL Lysis Buffer. Vortex for 30 seconds to mix thoroughly.

4. Let sit at room temperature for 5-10 minutes.

5. Centrifuge briefly to collect any liquid droplets from the lid.

6. Add 350 µL 100% ethanol. Vortex for 30 seconds to mix thoroughly.

7. Centrifuge briefly to collect any liquid droplets from the lid.

8. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® RNA Mini Column to the manifold.

9. Transfer 750 µL sample (including any precipitate) to the HiBind® RNA Mini Column.

10. Switch on vacuum source to draw the sample through the column.

    Note: If for any reason the solution has trouble passing through the column, turn off the vacuum, transfer the column to a 2 mL Collection Tube, centrifuge at maximum speed for 5 minutes or until all the sample passes through the column. Continue with Step 9 of the Centrifugation Protocol on Page 8.

11. Turn off the vacuum.

12. Repeat Steps 9-11 until all the lysate has been transferred to the HiBind® RNA Mini Column.

13. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

14. Switch on vacuum source to draw the VHB Buffer through the column.

15. Turn off the vacuum.

16. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

17. Switch on vacuum source to draw the RNA Wash Buffer II through the column.

18. Turn off the vacuum.

19. Repeat Steps 16-18 for a second RNA Wash Buffer II wash step.

20. Transfer the HiBind® RNA Mini Column to a new 2 mL Collection Tube.

21. Centrifuge the empty HiBind® RNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

22. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

23. Add 20-50 µL DEPC Water directly to the center of column matrix.

24. Centrifuge at maximum speed for 1 minute.

25. Store RNA at -70°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Problem in downstream applications	Salt carry-over during elution	• Ensure RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle • RNA Wash Buffer II must be stored at room temperature • Repeat wash with RNA Wash Buffer II
	PCR Inhibitors	• Dilute the starting sample with PBS Buffer
Problem	**Cause**	**Solution**
DNA contamination	DNA contamination	• Perform an on-membrane DNase digestion (Refer to Product# E1091 for more details)

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Vacuum Manifold	VAC-08
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
2 mL Collection Tubes	SS1-1370-00
DEPC Water, 100 mL	PR032
RNA Wash Buffer II, 50 mL	PR031
RNase-free DNase Set, 50 preps	E1091
RNase-free DNase Set, 200 preps	E1091-02